UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
Post Office Box 844
Ames, Iowa 50010

SAM - 801

9 CFR 113.51	September 16, 1983	Endogenous Viruses
Standard Requirement	Revised	Agent

SUPPLEMENTAL ASSAY METHOD

FOR

DETECTION OF PORCINE CELLS

FOR

PORCINE PARVOVIRUS CONTAMINATION

A. SUMMARY

This is an in vitro test procedure for examining porcine origin vaccine production cells for the presence of porcine parvovirus. The procedure uses several methods, e.g., immunofluorescence, hemagglutination, and aniline dye staining.

B. MATERIALS

1. Containers

Four or more sterile plastic or glass tissue culture containers having at least 75 cm_2 surface area, and at least one (1) rack of sterile Leighton tubes with coverslips or two flats of Tech slides (Miles Laboratories, Elkhart, IN. No endorsement expressed or implied).

2. Cell Culture

Enough production cells and media to seed two (2) containers from each production lot of cells to be tested.

3. Serum

A supply of pretested serum that is normally used by the manufacturer. In the case that porcine serum is used, it shall be negative by either serum neutralization (SN) hemagglutination inhibition (HI) for porcine parvovirus antibodies.

4. Medium

A supply of medium that is normally used to propagate production of swine cells and will grow a monolayer in 6 to 7 days.

5. Conjugate

An anti-porcine parvovirus conjugate. An initial supply will be furnished by the National Veterinary Services Laboratories for reference.

6. Stain

An aniline dye stain that will demonstrate intranuclear inclusion bodies, e.g., May Gruenwald-Giemsa of Shorr's.

7. Red blood cells

Guinea pig.

8. Control virus

A supply of pretitered porcine parvovirus. The seed will be furnished by the National Veterinary Services Laboratories.

C. METHOD

1. At least two (2) cell culture containers are seeded with each lot of porcine cells to be tested (a lot shall consist of equal aliquots of cells from pigs in one litter only). The trypsin used to disperse these cells shall be from a pretested negative lot or the trypsin shall have been sterilized with Beta-propiolactone. The cells are incubated at 35 to 37 C until a complete monolayer has formed.

2. After the monolayer has formed, remove the cells either with BPL treated trypsin in ATV or by scraping. The cells from the two flasks are pooled and redispersed in fresh growth medium. The cells are reseeded in fresh sterile containers at a 1:1 ratio, unless the manufacturer has a ratio that grows better in their experience. There shall be at least two containers to continue the test. Incubate as before in C.1.

3. At the time that the cell spread is essentially complete, the cells are removed from the containers as described under C.1. and redispersed (one container is reseeded into a sterile fresh container). At this time, the

equivalent of one container is seeded onto at least 20 Leighton tubes with slides or 10 Tech slides. At this time, at least ten (10) of these Leighton tubes or four Tech slides are inoculated with 0.1 ml of swine parvovirus diluted to contain approximately 100 $TCID_{50}$'s per tube. All vessels are incubated as before.

4. On the second day post-seeding, at least two Leighton tubes or one Tech slide from the test cell tubes and two Leighton tubes or one Tech slide from the positive control tubes are removed and fixed for fluorescent antibody staining. This procedure is repeated on the third, fourth, and fifth day. The slides may be stained every day or they may be held at -20 C until all have been fixed.

On the seventh day, the fluid from one container is removed and a hemagglutination test is conducted using the 1:2 through 1:32 dilutions. At least two Leighton tubes or one Tech slide of test cells and two positive control Leighton tubes or one Tech slide are removed on the first day the positive slides show fluorescence. The slides are fixed for aniline dye staining and stained.

D. Interpretation

1. The cells are satisfactory for freedom of porcine parvovirus if they do not fluoresce specifically after FA staining. The positive control cell shall stain positively.

The fluids taken for the HA test shall not show any hemagglutination.

The aniline dye stained coverslips shall not exhibit any intranuclear inclusion bodies. The positive control cells may or may not show inclusions.